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Elevation of Soluble CD23 in Sera From Patients With Infectious Mononucleosis

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CD23 is induced in B cells upon infection by Epstein-Barr virus (EBV) and a soluble form (soluble CD23: sCD23) is found in culture supernatants from EBV-transformed B cell lines. Based on these observations, we measured serum sCD23 levels in patients with infectious mononucleosis (IM) caused by EBV infection. Sera from patients with IM at the time of diagnosis contained more sCD23 than sera from normal control subjects. Changes in serum sCD23 levels during the course of disease showed that serum sCD23 levels were elevated at the time of diagnosis and they decreased to the normal levels during the convalescent phase defined by the improvement of symptoms of IM. These results indicate that the elevated levels of sCD23 were observed at the acute phase of IM and may be useful in diagnosing IM. ***J. Med. Virol. 53:384–387, 1997.*** © 1997 Wiley-Liss, Inc.

KEY WORDS: soluble CD23; infectious mononucleosis

INTRODUCTION

EBV is the etiologic agent of IM and is closely associated with the development of Burkitt's lymphoma and nasopharyngeal carcinoma [Epstein et al., 1979]. CD23, originally described as a B cell activation antigen, is identical to low affinity receptor for IgE (FcεRII), and is induced in B lymphocytes upon infection by EBV [Thorley-Lawson et al., 1985] and stimulation by cytokines such as interleukin-4 [Kawabe et al., 1988]. It is generally believed that sCD23 is produced, mainly, by the proleolytic cleavage of membrane CD23 [Letellier et al., 1990]. Soluble CD23 has been shown to be released in the culture supernatants of EBV-transformed B cell lines [Sarfati et al., 1984] and the elevation of sCD23 levels in plasma/serum from various diseases including EBV-related disorders [Yoshikawa et al., 1994]. However, little is known about serum levels of sCD23 in patients with IM. Therefore, we attempted to measure the amounts of sCD23 in sera from patients with IM in order to clarify CD23 expression in EBV-infectious disease, IM.

MATERIALS AND METHODS

The study group comprised 16 patients with IM (eight women and eight men) with mean age of 24.3 years (range 16 to 36 years) and 54 normal healthy control subjects with mean age of 34.1 years (range 26 to 45 years). The patients with IM and normal healthy control subjects had normal levels of serum IgE and no history of allergy. Informed consent was obtained from all patients and normal healthy control subjects. Clinical diagnosis of IM was confirmed based on fever, lymphadenopathy, splenomegaly, lymphocytosis, atypical lymphocytes in blood smears, positive serological tests for heterophile antibody, and the elevated levels of IgM antibody against EBV capsid antigen. The day of onset of IM was defined on basis of appearance of fever. Initial blood samples were taken at the time of diagnosis coming and as soon as possible after onset of IM. The period between the onset of IM and the day of collecting of blood samples varied with the patients [8.6 ± 6.6 days (mean ± SD); median 7 days (range 3 to 30 days)]. Serial blood samples were taken from five out of the 16 patients at the time of diagnosis and during convalescent phase. Convalescent phase was defined by the improvement of symptoms such as fever, lymphadenopathy, splenomegaly and by the absence of lymphocytosis, and atypical lymphocytes in blood smears. The determination of sCD23 molecules were made by enzyme-linked immunoabsorbent assay (ELISA). sCD23 molecules were evaluated quantitatively with a commercially available ELISA kit (Kurarey Co., Kurashiki, Japan). This kit utilizes a sandwich enzyme immunoassay using two monoclonal antibodies (H107 and E70) which recognized different epitopes of CD23

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to estimate serum sCD23 levels. ELISA was performed according to the manufacturer's instruction and as described elsewhere [Hashimoto et al., 1995]. Briefly, H107 was purchased from Nichirei Co. Ltd. (Tokyo, Japan) and E70 was purified from the ascites of BALB/c mice injected intraperitoneally with hybridoma producing monoclonal antibody to sCD23 by a Protein A immobilized agarose chromatography. An ELISA plate (Immunolon 4 flat bottom, Dynatech Laboratories, Alexandria, VA) was coated with E70. Nonspecific binding was blocked with 10 mM PBS (pH 7.2) containing 1% BSA and 0.05% Tween 20. sCD23 standards and samples which had been diluted with 20 mM Tris buffer (pH 7.5) containing 150 mM NaCl and 10% normal calf serum were applied in duplicate and incubated at 37°C for 2 hr. After incubation, the plate was washed with PBS and incubated with alkaline phosphotase-conjugated H107 at 37°C for 2 hr. The enzyme reaction was developed by addition of enzyme substrate solution (3.33 mg/ml p-nitrophenylphosphate solution dissolved in 1 mM diethannolamine (pH 10) containing 0.5 mM $MgCl_2$. After incubation at 37°C for 2 hr, color development was stopped by adding a stopper solution (0.2 N NaOH) and absorbance at 405 nm was measured in an ELISA reader. Statistical significance was analyzed using Mann-Whitney U-test. *P* values less than 0.05 were considered significant.

RESULTS

Sera from patients with IM at the time of diagnosis (within 30 days after onset of disease) contained more sCD23 (n = 16; range 1.16 to 8.36 ng/ml; mean ± SD 3.20 ± 1.88; median 3.30) than sera from normal subjects (n = 54; range 0.17 to 3.28; mean ± SD 1.33 ± 0.70; median 1.22) ($P < 0.01$); Fig. 1. Multiple serum samples were collected from five patients with IM to evaluate the changes in serum sCD23 levels relative to the course of this disease. Figure 2 shows serial changes in the levels of serum sCD23. Serum sCD23 levels were elevated at time of diagnosis and they decreased to the normal levels during the convalescent phase defined by the improvement of symptoms of IM. Relationship between serum sCD23 levels and clinical features of IM is shown in Table I In the six patients who had lower serum sCD23 levels than the mean plus 2SD of serum sCD23 levels in normal subjects, three (50%) and three (50%) had splenomegaly and lymphadenopathy, respectively. On the other hand, in the 10 patients who had higher serum sCD23 levels, nine (90%) and nine (90%) had splenomegaly and lymphadenopathy, respectively. These results indicated that the patients who had lower serum sCD23 levels than the mean plus 2SD of serum sCD23 levels in normal subjects had a milder disease.

We also measured serum sCD23 levels in other viral infections such as rubella and measles. Serum sCD23 levels in four patients with rubella with mean age of 18.8 years and in three patients with measles with mean age with 18.3 years were 1.18 ± 0.18 and 1.37 ± 0.21 ng/ml, respectively.

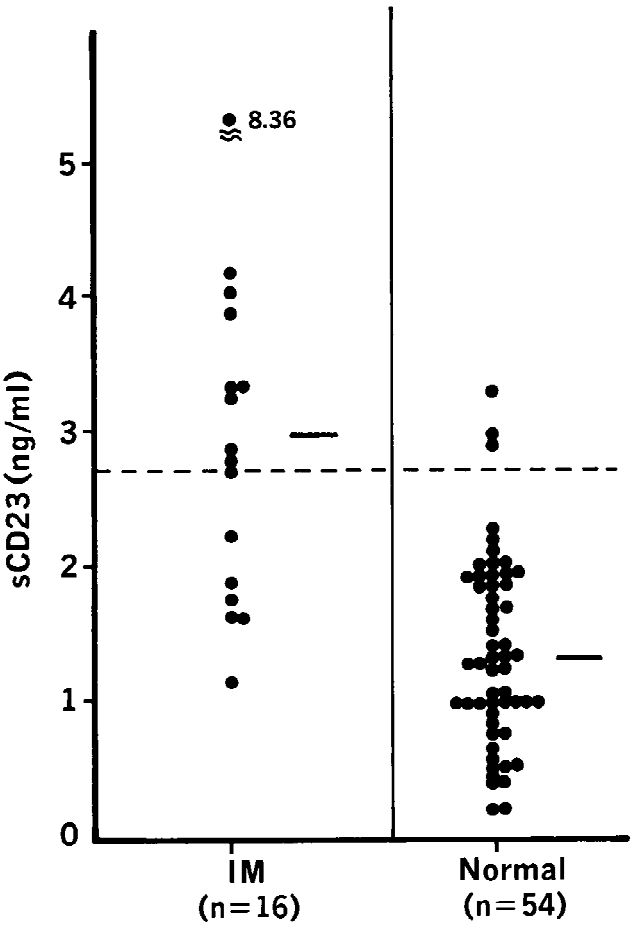

Fig. 1. Serum sCD23 levels in patients with IM and normal subjects. The horizontal short lines represent the mean of each group and the dashed line represents the mean plus 2SD of normal subjects.

DISCUSSION

sCD23 has been shown to be a regulatory molecule in immune responses and exerts various activities either alone or synergistically with cytokines, such as the promotion of B cell and T cell growth [Caris et al., 1990; Gordon et al., 1991; Lecron et al., 1991]. In addition, this molecule has growth factor-like activities on EBV-transformed B cells [Conrad, 1990; Yodoi et al., 1989]. The present results suggest that the elevated sCD23 may play a role in an intense T and B cell proliferation in IM, including activation of B cells which are not infected by EBV and could be the source of autoantibodies. A similar mechanism may account for the possible association of EBV with autoimmune diseases such as Sjögren's syndrome and rheumatoid arthritis, in which active EBV infection has been postulated [Saito et al., 1989; Takei et al., 1997]. Besides these stimulatory activities of sCD23 on lymphocytes, one could postulated that sCD23 may have a function to inhibit the infection of B cells by EBV. It has been shown that CD21, the receptor for EBV, is capable of binding sCD23 [Auby et al., 1992]. sCD23 released into serum of IM patients during acute phase of this disease

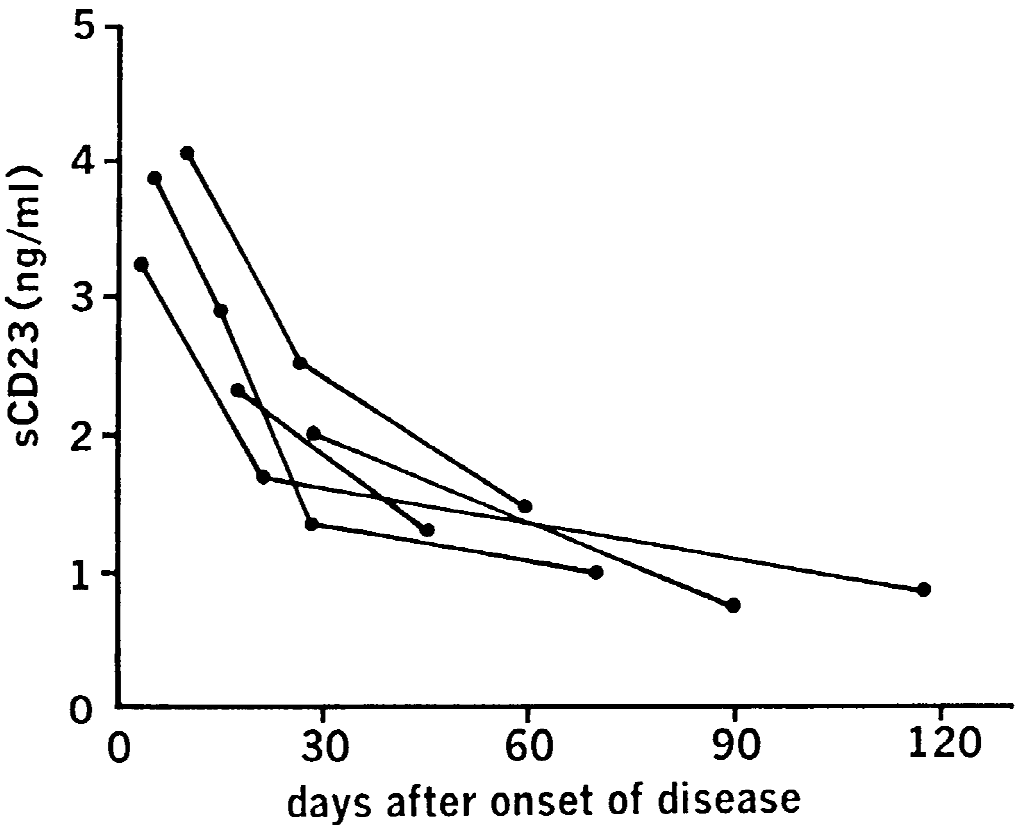

Fig. 2. Changes in serum sCD23 levels during the course of IM. Serial serum samples were obtained five out of the 16 patients with IM at the time of the diagnosis and during convalescent phase of disease to evaluate the relationship between serum sCD23 levels and clinical features of IM.

TABLE I. Relationship Between Serum sCD23 Levels and Clinical Features of IM

Clinical and laboratory findings	Serum sCD23 levels (ng/ml)	
	<2.73[a] n = 6[b]	>2.73 n = 10[b]
Fever (°C)	37.6 ± 0.32[c]	38.0 ± 0.29
CRP (mg/ml)	1.9 ± 0.44	2.8 ± 0.82
Atypical lymphocytes	6 (100)[d]	10 (100)
(%)	6.0 ± 1.4	7.1 ± 2.1
Splenomegaly	3 (50)	9 (90)[e]
Lymphadenopathy	3 (50)	9 (90)[e]
Days between the onset and the diagnosis of disease (days)	11.8 ± 9.9	7.5 ± 2.4

[a]2.73 ng/ml, the mean plus 2SD of normal subjects.
[b]In the 16 patients with IM, six patients had lower serum sCD23 levels than the mean plus 2SD of serum sCD23 levels in normal subjects, whereas 10 patients had higher serum sCD23 levels than the mean plus 2SD of those.
[c]Mean ± SD.
[d]Number (%) of patients. CRP, C-reactive protein.
[e]$p < 0.01$, as evaluated by Fisher's exact probability.

may compete with EBV for its receptor CD21 on B cells. Thus, the binding of sCD23 to CD21 may inhibit the binding and subsequent infection of B cells by EBV.

Our results showed that serum sCD23 levels are elevated at the acute phase of IM and they decreased to the normal levels during convalescent phase. We also measured serum sCD23 levels in patients with rubella and patients with measles, however; their serum sCD23 levels were compatible to normal subjects. We did not measure serum sCD23 levels in other lymphotropic virus-infectious disease such as cytomegalovirus (CMV) infection, however; there is no evidence for CMV-induced up-regulation of CD23 expression in lymphocytes in the literature.

In conclusion, our study demonstrated that the elevated levels of serum sCD23 were observed at the acute phase of IM. Biological actions of sCD23 have been shown, however; their role in IM remains to be clarified.

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